SYNOPSIS

GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES FROM *ECLIPTA PROSTRATA* AGAINST ORAL PATHOGENS AND ORAL MATRIX METALLOPROTEINASES

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By

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STUDIES ON EFFECT OF ZINC OXIDE NANOPARTICLES FROM *ECLIPTA PROSTRATA* AGAINST ORAL PATHOGENS AND ORAL MATRIX METALLOPROTEINASES

INTRODUCTION

Nanotechnology involves the use of materials having nanoscaledimensions in the range of 1–100 nm. Operating with nanomaterials has allowed researchers to have a much better understanding of

biology. The green synthesis of nanoparticles has greatly reducedthe use of physical and chemical methods. Various chemical methods have been proposed for the synthesis of zinc oxide nanoparticles (ZnO NPs), such as reaction of zinc with alcohol, vapor transport, hydrothermal synthesis, precipitation method etc (Banerjee and Narendrakannan,2011) . Theuse of green synthesis method by the researchers is rapidly increasing due to usage of less toxic chemicals, eco-friendly natureand one step synthesis of nanoparticlesinvolved in the green synthesis from plants andtheir derivatives, microorganisms like bacteria, fungi.

ECLIPTA PROSTRATA

Taxonomic Tree

- Domain: Eukaryota
- Kingdom: Plantae
- Phylum: Spermatophyta
- Subphylum: Angiospermae
- Class: Dicotyledonae
- Order: Asterales
- Family: Asteraceae
- Genus: Eclipta
- Species: Prostrata

ORAL PATHOGENS

The oral microbiome, mainly comprising bacteria which have developed resistance to the human immune system, has been known to impact the host for its own benefit, as seen with **dental cavities.** The environment of the human mouth allows the growth of characteristic microorganisms found there. It provides a source of water and nutrients, as well as a moderate temperature. Resident microbes of the mouth adhere to the teeth and gums to resist mechanical flushing from the mouth to stomach where acid-sensitive microbes are destroyed by hydrochloric acid (*Santhosh kumar.et.al*,2017).

Anaerobic bacteria in the oral cavity of the human beings most commonly are bacteria which include: *Actinomyces*, *Arachnia*, *Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Fusobacterium*, *La ctobacillus*, *Leptotrichia*, *Peptococcus*, *Peptostreptococcus*, *Propionibacterium*, *Selenomonas*, *T reponema*, and *Veillonella*.In addition, there are also a number of fungi found in the oral cavity, which include*Candida, Cladosporium, Aspergillus, Fusarium, Glomus, Alternaria, Penicillium*, and *Cryptococcus*. The oral cavity of a new-born baby does not contain bacteria but rapidly becomes colonized with bacteria such as *Streptococcus salivarius*. With the appearance of the teeth during the first year colonization by *Streptococcus mutans* and *Streptococcus sanguinis* occurs as these organisms colonise the dental surface and gingiva. Other strains of streptococci adhere strongly to the gums and cheeks but not to the teeth. The gingival crevice area (supporting structures of the teeth) provides a habitat for a variety of anaerobic species. Bacteroides and spirochetes colonize the mouth around puberty (*Santhoshkumar et.al* , 2017). Of particular interest is the role of oral microorganisms in the two major dental diseases: dental caries and periodontal disease.

The habitat of the oral microbiome is essentially the surfaces of the inside of the mouth. Saliva plays a considerable role in influencing the oral microbiome. More than 800 species of bacteria colonize oral mucous, 1,300 species are found in the gingival crevice, and nearly 1,000 species comprise dental plaque. The mouth is a rich environment for hundreds of species of bacteria since saliva is mostly water and plenty of nutrients pass through the mouth each day. When kissing, it takes only 10 seconds for no less than 80 million bacteria to be exchanged by the passing of saliva. However, the effect is transitory, as each individual quickly returns to their own equilibrium.

Thanks to progress in molecular biology techniques, scientific understanding of oral ecology is improving. Oral ecology is being more comprehensively mapped, including the tongue, the teeth, the gums, salivary glands, etc. which are home to these communities of different microorganisms.

The host's immune system controls the bacterial colonization of the mouth and prevents local infection of tissues. A dynamic equilibrium exists notably between the bacteria of dental plaque and the host's immune system, enabling the plaque to stay behind in the mouth when other biofilms are washed away. In equilibrium, the bacterial biofilm produced by the fermentation of sugar in the mouth is quickly swept away by the saliva, except for dental plaque. In cases of imbalance in the equilibrium, oral microorganisms grow out of control and cause oral diseases such as tooth decay and periodontal disease. Several studies have also linked poor oral hygiene to infection by pathogenic bacteria (*Bala et.al*.,2015)

There are many factors of oral health which need to be preserved in order to prevent pathogenesis of the oral microbiome or diseases of the mouth. Dental plaque is the material that adheres to the teeth and consists of bacterial cells (mainly *S. mutans* and *S. sanguis*), salivary polymers and bacterial extracellular products. Plaque is a biofilm on the surfaces of the teeth. This accumulation of microorganisms subjects the teeth and gingival tissues to high concentration of bacterial metabolites which results in dental disease. If not taken care of, via brushing or flossing, the plaque can turn into tartar (its hardened form) and lead to gingivitis or periodontal disease. In the case of dental cavities, proteins involved in colonization of teeth by *Streptococcus mutans* can produce antibodies that inhibit the cariogenic process which can be used to create vaccines. Bacterial species typically associated with the oral microbiota have been found to be present in women with bacterial vaginosis. Genera of fungi that are frequently found in the mouth include *Candida*, *Cladosporium*, *Aspergillus*, *Fusarium*, *Glomus*, *Alternaria*, *Penicillium*, and *Cryptococcus*, among others. Additionally, research has correlated poor oral health and the resulting ability of the oral microbiota to invade the body to affect cardiac health as well as cognitive function. High levels of circulating antibodies to oral pathogens *Campylobacter rectus*, *Veillonella parvula* and *Prevotella melaninogenica* are associated with hypertension in human (*Nayak et.al*.,2011).

Most of the bacterial species found in the mouth belong to microbial communities, called biofilms, a feature of which is inter-bacterial communication. Cell–cell contact is

mediated by specific protein adhesins and often, as in the case of inter-species aggregation, by complementary polysaccharide receptors. Another method of communication involves cell–cell signalling molecules, which are of two classes: those used for intra-species and those used for inter-species signalling. An example of intra-species communication is quorum sensing. Oral bacteria have been shown to produce small peptides, such as competence stimulating peptides, which can help promote single-species biofilm formation (Bentes ,2003). A common form of inter-species signalling is mediated by 4, 5-dihydroxy-2, 3-pentanedione (DPD), also known as autoinducer-2 (Al-2) (*Gunalan et.al.,2012*).

The microorganisms found in the human oral cavity have been referred to as the oral microflora, oral microbiota, or more recently as the oral microbiome. The term microbiome was coined by Joshua Lederberg "to signify the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space and have been all but ignored as determinants of health and disease". The term microbiome has been embraced by the Human Microbiome Project and investigators who believe an understanding of human health and disease is impossible without fully understanding the collective microbiome/human "superorganism." Their work describe the identification and phylogeny of the most prevalent oral taxa. The oral cavity, or mouth, includes several distinct microbial habitats, such as teeth, gingival sulcus, attached gingiva, tongue, cheek, lip, hard palate, and soft palate. Contiguous with the oral cavity are the tonsils, pharynx, esophagus, Eustachian tube, middle ear, trachea, lungs, nasal passages, and sinuses. The human oral microbiome is defined as all the microorganisms that are found on or in the human oral cavity and its contiguous extensions (stopping at the distal esophagus), though most of our studies and samples have been obtained from within the oral cavity (*Preshaw et.al.,*2004).

MATRIX METALLOPROTEINASES

Matrix metalloproteinases (MMP) also known as matrixins, are calcium-dependent, zinc-containing endopeptidases. Collectively these enzymes are capable of degrading all kinds of Extra Cellular Matrix (ECM) proteins, but can also process a number of bioactive molecules. They are known to be involved in cleavage of cell surface receptors, the release of apoptotic ligands and chemokine/cytokine inactivation.

General domains of the *MMP* protein

The *MMP* protein has three main domains, namely:

- Pro-peptide domain.
- Peptidase domain.
- Hemopexin domain.

Humans have 24 *MMPs* with overlapping functions making it much too complex and difficult for us to perform studies on human subjects.

The most commonly used groupings (by researchers in MMP biology) are based partly on historical assessment of the substrate specificity of the MMP and partly on the cellular localization of the MMP. These groups are the collagenases, the gelatinases, the stromelysins, and the membrane-type MMPs (MT-MMPs) (*Abraham et.al.,2005*).

- The collagenases are capable of degrading triple-helical fibrillar collagens into distinctive 3/4 and 1/4 fragments. These collagens are the major components of bone, cartilage and dentin, and MMPs are the only known mammalian enzymes capable of degrading them. The main collagenases are No. 1, No. 8, No. 13, and No. 18. In addition, No. 14 has also been shown to cleave fibrillar collagen, and there is evidence that No. 2 is capable of collagenolysis. In MeSH, the current list of collagenases includes No. 1, No. 2, No. 8, No. 9, and No. 13. Collagenase No. 14 is present in MeSH but not listed as a collagenase, while No. 18 is absent from MeSH.
- The main substrates of the gelatinases are type IV collagen and gelatin, and these enzymes are distinguished by the presence of an additional domain inserted into the catalytic domain. This gelatin-binding region is positioned immediately before the zinc-binding motif, and forms a separate folding unit that does not disrupt the structure of the catalytic domain. The gelatinases are No. 2 and No. 9.

- The stromelysins display a broad ability to cleave extracellular matrix proteins but are unable to cleave the triple-helical fibrillar collagens. The three canonical members of this group are No. 3, No. 10, and No. 11.
- All six membrane-type MMPs (No. 14, No. 15, No. 16, No. 17, No. 24) have a furin cleavage site in the pro-peptide, which is a feature also shared by No. 11.

OBJECTIVES

- Green synthesis of nanoparticles using eco-friendly, non-toxic method which has less usage of chemicalscompared to physical and chemical method.
- Sub-culturing of oral pathogens , mainly seen in the oral cavity of periodontitis patients.
- Extracting of matrix metalloproteinases mainly salivary gelatinases from the saliva of periodontitis patients using zymography technique.
- Analyzing the inhibiting effect of nanoparticles on the growth of bacteria as well as on matrix metalloproteinases

EXPERIMENTAL DESIGN

The leaves of *Eclipta prostrata* were collected from Koyambedu market, chennai.It is a medicinally important plant. Fresh green leaves were harvested during the months of September to January. 500g of fresh leaves was washed with running tap water followed by Milli-Q water and then cut, and soaked in a 250 mL Erlenmeyer flask containing 100 Ml Milli-Q. The solution was boiledat 70 ℃ for 8 min. The leaf extract was allowed to cool to roomtemperature, filtered through Whatman number-1 filter paper, andthe filtrate was stored for further experimental use.

- Green synthesis of zinc oxide nanoparticles were done using zinc acetate standard method. And the biosynthesized particles were analyzed using UV – visible range and XRD spectra.

- The antibacterial activity of synthesized ZnO NPs was performed by agar well diffusion method against laboratory strains of oral pathogens*Lactobacillus casei,Streptococcus mutans,Enterococcus feacalis and Candida albicans* .

- Extraction of MMP-1 from human saliva of periodontitis patient.

- Inhibition of MMP-1 by zinc nanoparticles of *Eclipta prostrata*.

MAJOR OUTCOMES

- As a preliminary confirmation, the rapid synthesis of ZnO NP wasmeasured using the UV- Visible spectroscopy at a maximum absorbance of 380 nm. Further the XRD analysis proved the crystalline nature of the ZnO NP, while these analysis confirmed thepresence of zinc and oxide ions in the nanoparticles (figure:1).

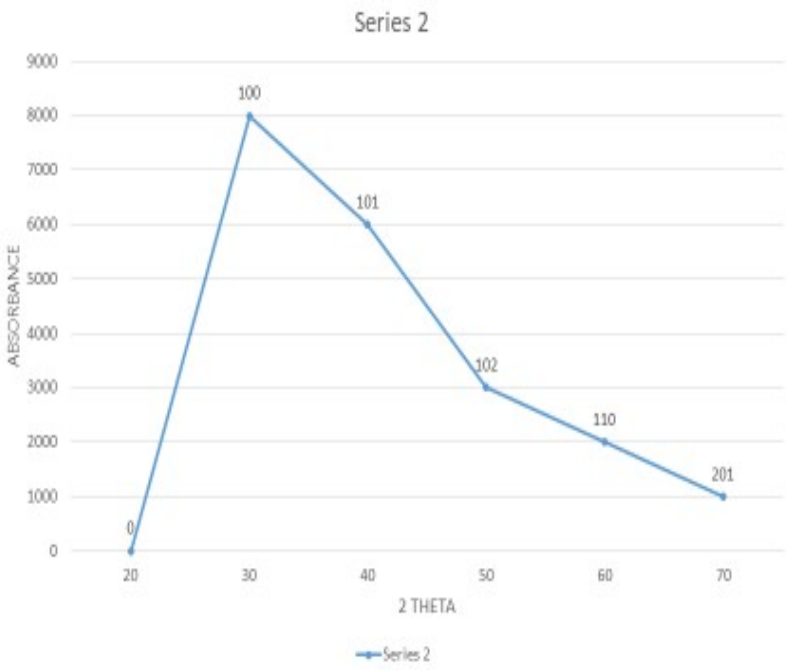

Fig:1-:- XRD spectrum of ZnO NPs

- UV-visible spectroscopy is conducted to confirm the synthesis of ZnO NPs. Conducting electrons start oscillating at a certain wavelength range due to surface plasmon resonance (SPR)effect.The UV-visible spectra of freshly prepared ZnO NPs are given in figure : 2. Peak obtained at 600 nm clearly demonstrates the presence of ZnO NPs in the reaction mixture.

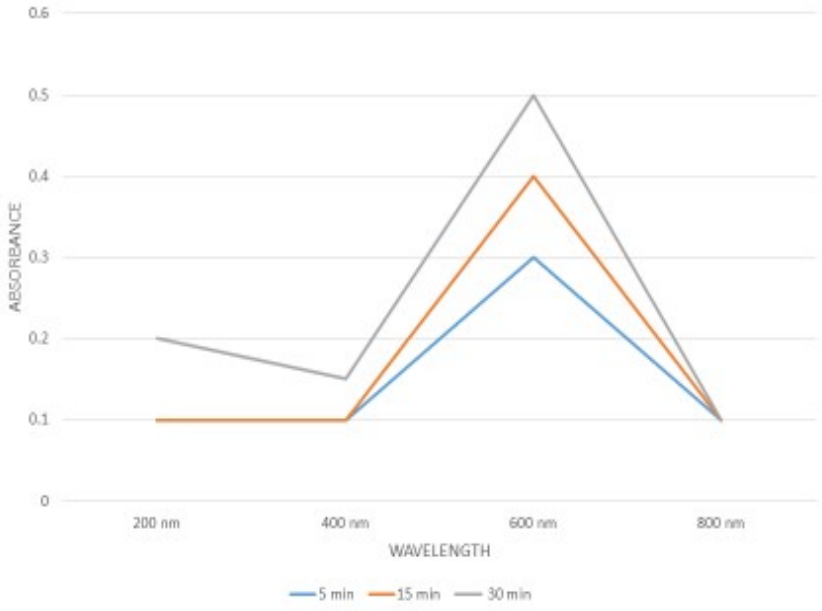

Fig 2 :Ultra-Violet Visible Spectrum of ZnO NPs synthesized by *E. prostrata*

- Also the anti-bacterial activity of ZnO NPs has proved that these can be used as potent anti-bacterial agent against oral microbial infection.

Particulars	Doxycycline	ZnO NPs
Range	78-86	30-80
Mean	82.1	50.5
Standard deviation	2.08	16.80

Figure : 3 :- Inhibition values

- On the basis of the current trial on MMP-1 inhibition , it can be stated that ZnO NPs achieved inhibition of MMP-1 at 75ul concentration, very much similar to that of doxycycline which is a conventional drug of choice.. The mean values showed an 82.1% reduction in the MMP-2 activity with the addition of doxycycline, 50.5% reduction in the MMP-1 activity .

ZONE OF INHIBITION

microorganism	ampicillin	25ul	50ul	75ul
Lactobacillus casei	11mm	09mm	10mm	11mm
Streptococcus mutans	12mm	08mm	11mm	12mm
Enterococcus feacalis	13mm	10mm	11mm	13mm

Figure :- 4 : Zone of inhibition

The detailed experimental procedure and results will be discussed in the thesis with relevant literature.

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